WOOD-DECOMPOSING FUNGI AND THEIR ADAPTATION TO LIFE IN WOOD*

Dr. JOSTEIN GOKSØYR *Botanical Laboratory, University of Bergen, Norway*

ONE OF THE MOST SERIOUS drawbacks to the use of wood as a construction material, is that under favourable conditions it easily decays and thus loses its strength and elasticity. This decay annually destroys wood worth large sums of money. In Norway, a rough estimate a few years ago set the value of constructional timber destroyed by rot at four times that destroyed by fire (£20 million against £5 million per year). In addition the destruction of standing forest trees, caused by heart rot and root rot, produces additional losses which certainly amount to very high values.

Wood decay thus represents a very serious economical problem, which requires us to be extremely interested in its prevention. However in doing so, we should not forget that wood decomposition is a chain in the circulation of carbon compounds on the earth, and in fact is quite an important chain, considering the huge amount of woody material that is produced annually on this planet.

Decomposition of wood is practically exclusively a biological process, caused by organisms that digest the wood and use it as a nutrient. However, the number of organisms which are able to do this is quite restricted. When it comes to real wood as in the trunk of trees, bacteria are of little or no importance. Besides some groups of animals (among which termites and wood-boring mussels (*Teredo*) are perhaps the most important), wood is digested practically solely by Hymenomycete fungi. Outside of this group we also find a few wood-decomposers among the Ascomycetes and Fungi Imperfecti.

If we divide the Hymenomycetes according to their manner of living, we find three major groups, which are all related to trees.

In the first group we have those that live inside the wood either in living or dead trees (or in constructional timber), and which cause rot by digesting the wood. In the second group there are those fungi that live in and decompose litter, that is, the layer of leaves, needles and small branches which fall from trees in a forest and cover the soil. In the third group we have those fungi that live in symbiosis with the trees, their mycelium engaging with the roots of the trees and forming mycorrhiza.

It is tempting to ask what is the reason for this differentiation, and what is it that causes a wood-decomposing fungus to prefer wood, and a litter-decomposing to prefer litter. It is my intention to consider this problem especially with regard to the wood-decomposing fungi.

The wood, and how it is attacked

Let us start with a brief look at the living-place of the wood-decomposing fungi. Wood is built up of fairly thick-walled cells. Most of the cells, even in a living tree, are dead and serve as a water-conducting system. When the tree is felled and dried, these cells become filled with air. Interspersed among these dead cells there is also a system of living cells, especially in the outer (younger) part of the trunk. These cells contain protoplasm, and will furnish the wood with a small amount of protein and all the other components present in a cell.

In the wood, the Hymenomycetes normally live within the dead cells, and they extend their territory by the growth of their hyphae, which can penetrate the cell walls. Figs. 1–2, made by Hartig[1] in 1878, may serve as an illustration of how the wood is attacked, and the cell walls decomposed. Fig. 3,

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* Paper delivered to Section K (Botany) and Section K* (Forestry) on August 28, 1964, at the Southampton Meeting of the British Association.

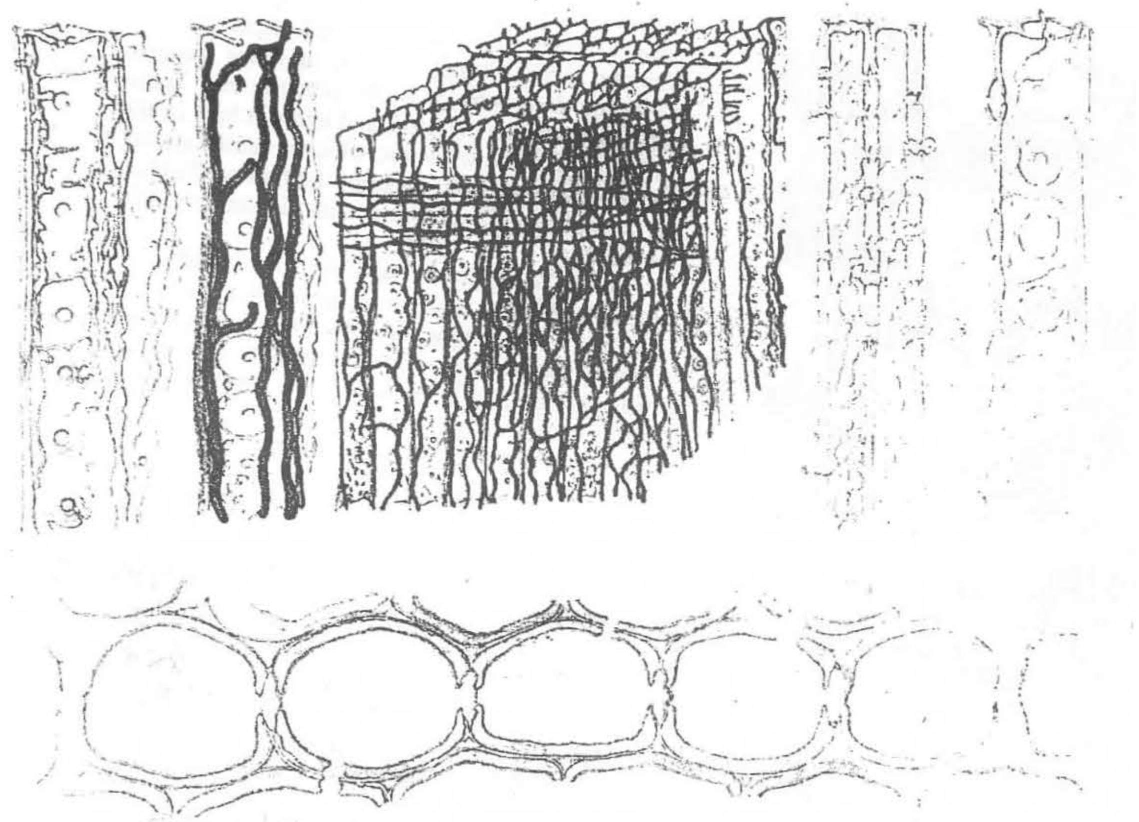


FIG. 1. Pine wood, attacked by *Trametes pini*, causing white rot. After Hartig.[1]

drawn freely after Nutman,[2] shows how the hyphae penetrate the cell walls. The first kind of penetration seems to be the most common. Even though the holes made by the hyphae are often called boreholes, there is every reason to believe that they are made enzymatically.

This leads us to a brief survey of the chemistry of wood, which is very complex, and still far from being completely elucidated. The main components in wood belong to two quite different groups of organic compounds. One is made out of the polysaccharides, and has long, thread-like molecules. Among these, cellulose is the most prominent, making up say 50–60 per cent of the wood mass. Cellulose is a polymer of β-glucose units (Fig. 4). Besides cellulose, wood also contains other polysaccharides, with glucose, other hexoses, or pentoses as building units. These polysaccharides are often given the common name hemicellulose. They make up 10–25 per cent of the wood mass; the lower value being normal for coniferous woods.

The second group of compounds is lignin, the incrusting substance, which we can say makes wood what it is. Lignin is an amorphous polymer of coniferyl alcohol or -aldehyde. Coniferyl alcohol contains a benzene nucleus, and this gives lignin an aromatic character. Between the building units there are a whole number of alternative·bridging bonds. These do not appear in a repetitive sequence, and this is the reason why lignin is amorphous. The molecular weight is very high, probably above 180,000. What we see on Fig. 5 is not a lignin molecule, but it is intended to give an idea of how such a molecule can be built up. We should notice the *R* in

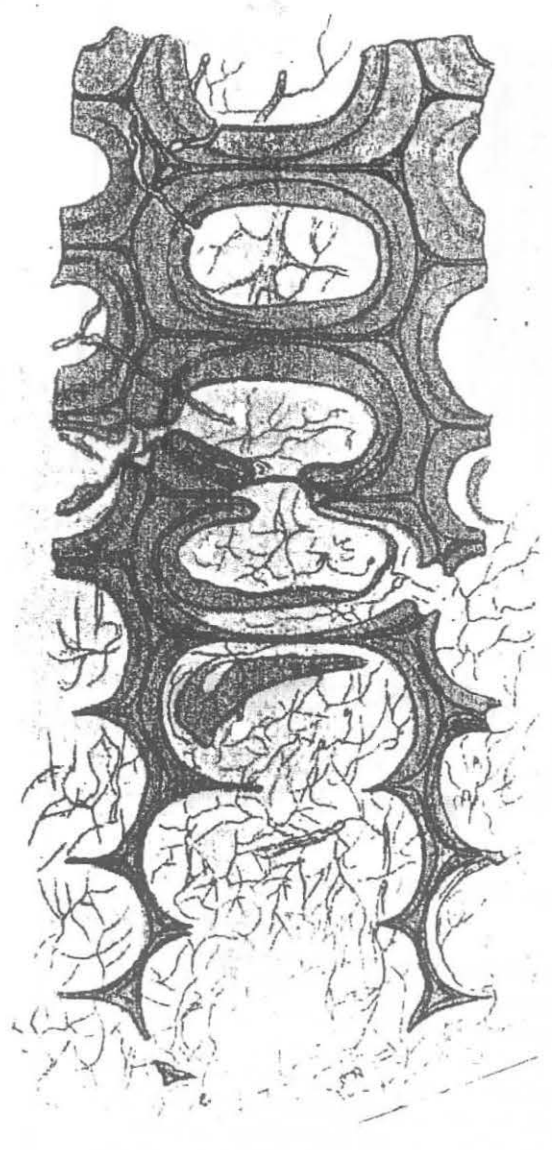


FIG. 2. A series of tracheids from pine wood, attacked by *Polyporus borealis*. After Hartig.[1]

the upper right side of the picture. What this may mean, we shall return to later.

The wood cell wall is not homogenous. It consists of a number of different layers, as we can see in Fig. 6. These layers have a different ratio between lignin and cellulose, the degree of lignification being highest in the middle lamella and the primary cell wall.

There are two main types of rot (together with intermediate types). In the type of rot which is called brown rot or cubic rot, and which is caused (among other fungi) by the dry rot fungus, *Merulius lacrymans*, it is the cellulose and hemicellulose that are digested, and lignin is left. In the other type of rot, white rot, which is caused among other fungi by *Fomes annosus*, the root rot fungus, it is lignin and hemicellulose that is preferentially attacked.

On Fig. 7 are shown some electron micrographs, made by Meier,[3] which demonstrate how *Merulius*, causing brown rot, has attacked the secondary wall, leaving the primary wall seemingly intact, while the white rot fungus *Trametes pini* has dissolved away the primary wall.

One interesting problem in wood chemistry, and very pertinent to us, is whether the polysaccharides in wood (cellulose and hemicellulose) are chemically linked to lignin. A glucosidic or anhydridic-bonding is theoretically quite possible, and the Freudenberg group in Germany, which has contributed a great deal to our knowledge of lignin chemistry and biosynthesis, seems now to be in favour of the existence of chemical bonds between lignin and the polysaccharides.[4] On Fig. 5, *R* could symbolize such a polysaccharide chain.

It is a well established fact that wood is attacked with greater difficulty by cellulose-decomposing organisms than pure cellulose. In particular we may note that several bacteria and fungi which can live on cellulose, evidently cannot digest wood. This may be simply because the cellulose is less accessible in the wood, surrounded as the cellulose fibrils are by lignin. But it may also be caused by the chemical bonds between lignin and cellulose, which a number of cellulose-digesting organisms would not then have the ability to break.

Perhaps mostly as a curiosity, we may note that Czapek[5] in 1899 reported an enzyme in *Merulius lacrymans*, capable of liberating easily extractable lignin from finely ground wood. At this time, not much was known about the chemistry of lignin. But the phloroglucinol-HCl reaction (giving a red colour with lignin, in fact with the coniferyl aldehyde groups) was known, and it was this reaction that Czapek utilized. Czapek purified his enzyme by ethanol precipitation (which by the way

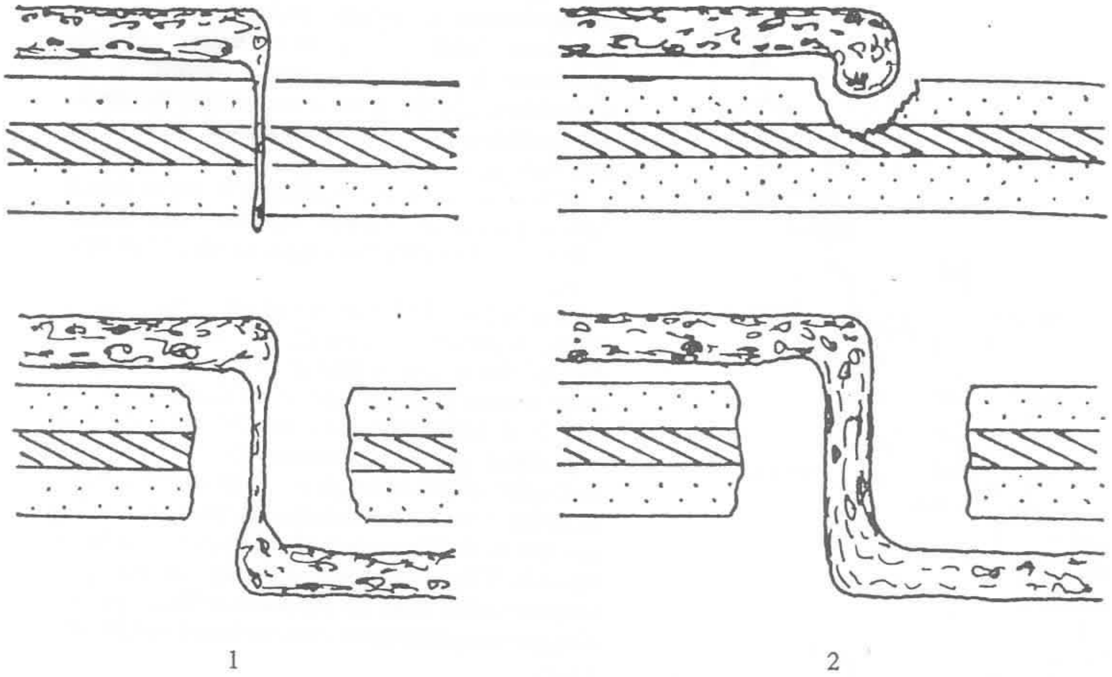


FIG. 3. The fungal hyphae may penetrate the cell walls either by a very fine thread as in 1, or by enzymatic dissolution of the cell wall in front of an ordinary hyphal tip, as in 2. In both cases, the hole is later extended.

indicates that it must have been quite stable), found that it was not a β-glucosidase, and named it hadromase after hadromal, which was what the carrier of the lignin reaction was then called. Other workers on Hymenomycete enzymology at the beginning of this century also reported on the presence of hadromase in brown rot fungi, but later the 'enzyme' seems to have been more or less forgotten.

In later years, Nord and collaborators[6] have substantiated the fact that even brown rot fungi, which do not change the amount of lignin in the wood, and which we normally say do not attack lignin, cause a great part of the lignin to become easily extractable. The lignin that can be extracted from brown-rotton wood differs, however, quite considerably from intact lignin. It has a much lower molecular weight and a lower methoxyl content. In my opinion, this is of more interest to lignin chemists. From our point of view the fact that something happens with the lignin even in brown rot is essential, and the question which we have to ask is whether that which happens is necessary for making cellulose and hemicellulose accessible, and

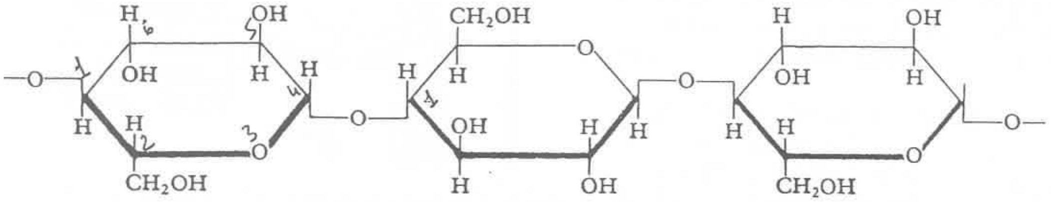


FIG. 4. Part of a cellulose molecule, with three glucose units. The complete molecule may contain more than a thousand glucose units.

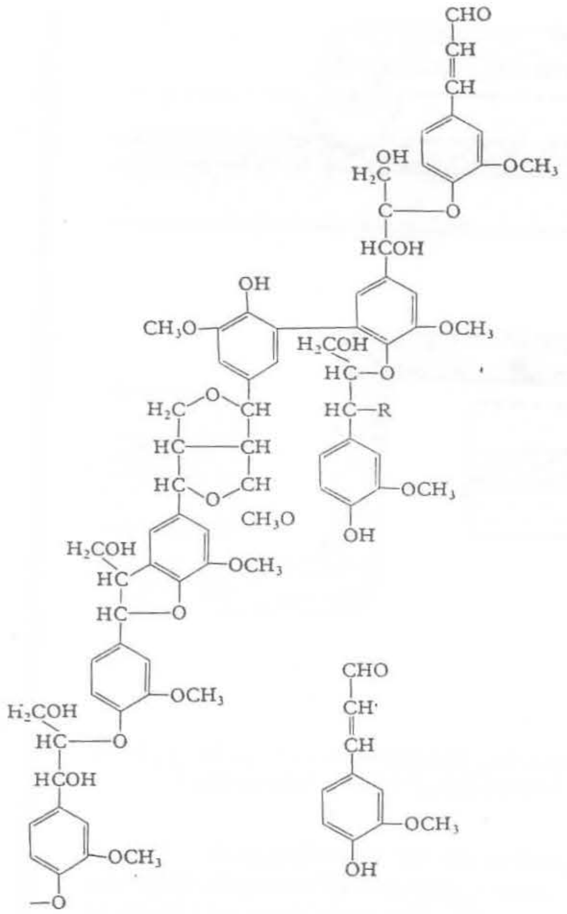


FIG. 5. What a small bit of a lignin molecule may look like. Inserted is coniferyl aldehyde, the building unit (monomer) of lignin.

if it is caused by a special enzyme system. If this is so, we can more easily understand the difference between the brown rot fungi and the plain cellulose decomposing micro-organisms.

We shall now turn briefly to that part of the enzyme equipment which the wood-rotting fungi need to *digest* the different components of the wood in order to be able to utilize them as carbon and energy sources.

Cellulose and hemicellulose are broken down hydrolytically by means of enzymes which are excreted by the fungal cells. Cellulase, which is the enzyme among these that has been best studied, breaks the glucosidic bonds in the cellulose chain more or less at random, so that the degree of polymerization is rapidly reduced, and finally cellobiose (with two glucose units) appears. Cellobiose is split by another enzyme, a β-glucosidase, and the glucose then enters the metabolic pathway which furnishes the cell with energy and building stones.

The white and intermediate rot types attack lignin, practically always together with hemicellulose, the intermediate types also together with cellulose.

How lignin is broken down and digested by these fungi, is partly understood. We owe to Bavendamm[7] the discovery that all lignin-decomposing fungi excrete phenolic oxidase, of the laccase type, and it is commonly believed that this enzyme degradates the lignin oxidatively, so that low-molecular weight aromatic compounds are formed. In order to become metabolized, the aromatic ring systems in the fragments thus formed have to be opened. This is probably done by the action of enzymes other than the phenolic oxidase. In fact, ring-opening enzymes have been found in white rot fungi.

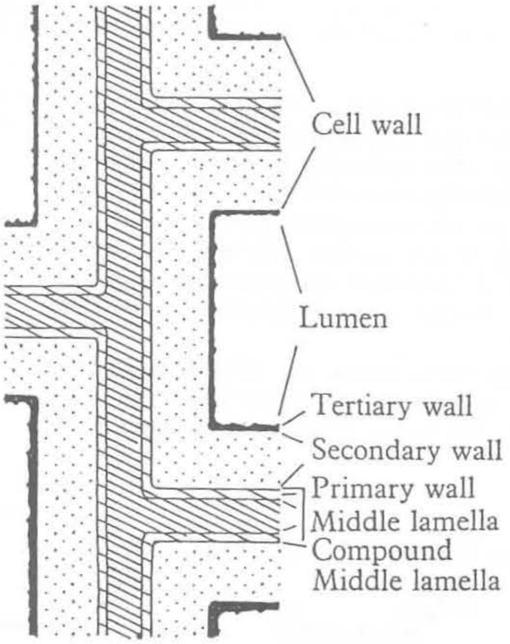


FIG. 6. Schematic drawing of cells in wood, demonstrating the layers in the cell wall.

Nitrogen and thiamin

The carbon compounds in the wood furnish the cell with energy and metabolites. But a growing organism cannot live on carbon and energy alone. It also needs a number of other nutrients. Of these, the mineral nutrients such as P, K, Mg, are found in the wood in ample amounts and remain in the ash when wood is burned. The nitrogen supply is more interesting. Coniferous woods may contain down to 0·04 per cent N. This is roughly 1000 times less than the carbon content of the wood.

Findlay[8] made a thorough investigation of the nitrogen utilization of wood-rotting fungi. He found that even though these fungi are extremely efficient in the utilization of nitrogen, many kinds of wood contain too little nitrogen to permit optimal growth. Hungate[9] has also discussed the nitrogen utilization of wood-decomposing fungi, and calculated that the quantity of carbon which they are able to decompose is almost 700 times the amount of nitrogen utilized. It is worth while considering this value in connection with the carbon/nitrogen ratio of 30 which Heukelekian and Waksman (1925) found for cellulose decomposing microfungi in soil. It certainly points towards extreme efficiency in nitrogen utilization being an important character in wood-inhabiting fungi.

To illustrate this further, we may imagine the large volumes of wood that have to be emptied of nitrogen when a fruiting body is formed. The nitrogen content of the wood is most probably due to proteins and amino acids. At least, wood-rotting fungi contain efficient proteolytic enzymes. This was shown as early as 1901 by Kohnstamm,[10] which was only a few years after Buchner's classical experiments with yeast maceration juice (the experiments which started modern biochemistry). Kohnstamm used the same technique

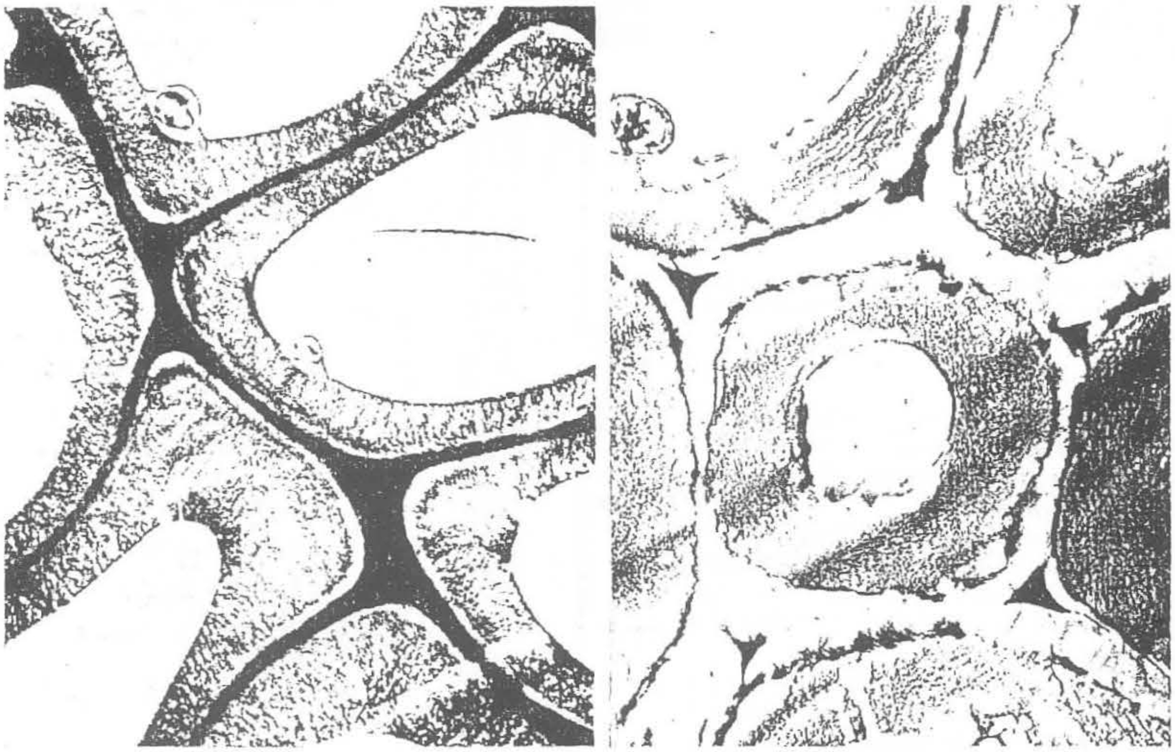
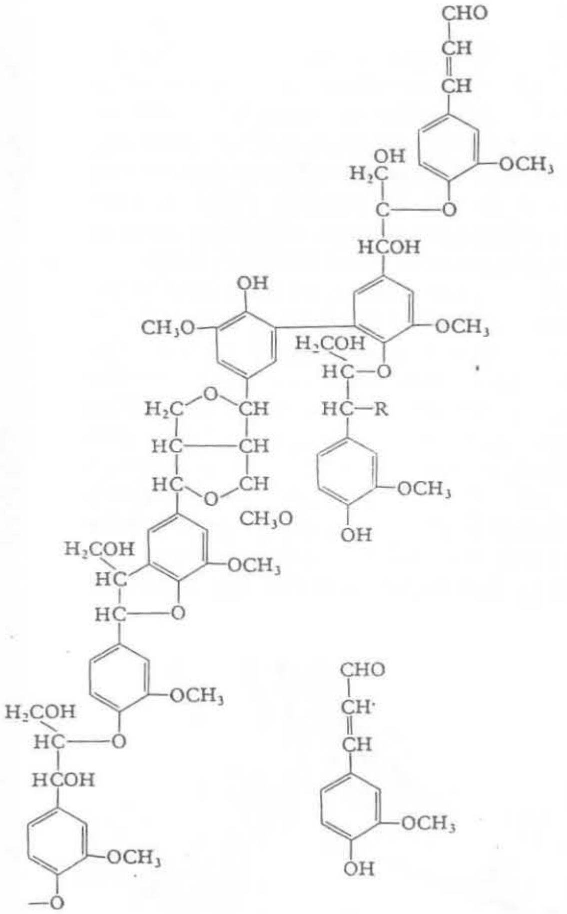


FIG. 7. Electron micrographs of wood attacked by fungi. (*a*) Pine wood after five months of attack by *Merulius lacrymans*. Note dissolving of the secondary wall. (*b*) Birch wood after eight months of attack by *Trametes pini*. The middle lamella and primary wall is dissolved. In both pictures, cross-sections of hyphae can be seen within the cells. After Meier.[3]



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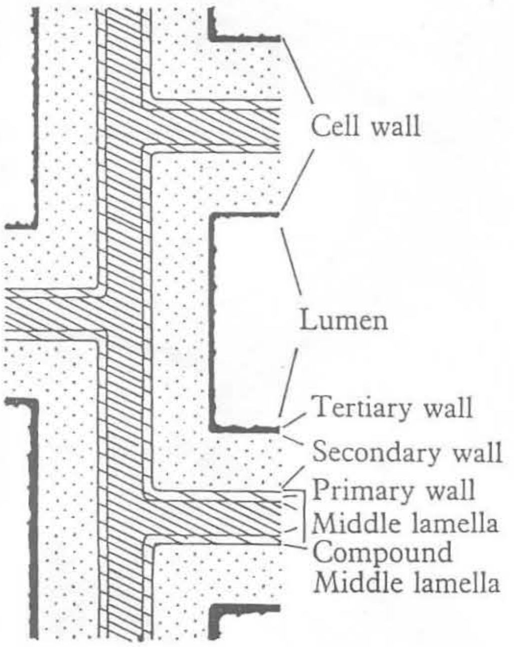

FIG. 6. Schematic drawing of cells in wood, demonstrating the layers in the cell wall.

organisms, either totally as in the obligate anaerobes, or during periods of oxygen shortage as in the facultative anaerobes. Facultative anaerobic fungi are known, the yeasts are somewhat specialized, but facultative anaerobes can also be found among the filamentous fungi.

One might think that to be a facultative anaerobe would be almost ideal for a wood-destroying fungus. However, we must also consider that, contrary to most other micro-organisms, such fungi live in an aqueous phase of small capacity, in which organic metabolic end products would soon accumulate to high concentrations. If the end produce of the fermentation is toxic, as it is in ordinary alcohol fermentation, it would thus be quite risky for the fungus. There are no indications that wood-rotting Hymenomycetes have fermentative capacities, as such are normally considered. I should perhaps add the reservation that an eventual formation of glycerol ought to be more closely studied.

When living on a minimum supply of oxygen, these fungi seem rather to burn the glucose incompletely and at a reduced rate, so that organic acids accumulate. Most of these fungi are in fact prominent acid producers, and some (but not all) can tolerate quite low pH values. Among the acids formed, oxalic acid is perhaps the dominating one, but citric acid is often found also. In fact, Smith[14] has stated that oxalic acid is the normal end product of metabolism in *Merulius lacrymans*, replacing carbon dioxide in other organisms. This is probably a quite considerable overestimate of the role of oxalic acid, at least according to our own findings.

We have carried out studies in our laboratory on the energy metabolism of *Merulius* in more detail than it is possible to go into here, but I would like to stress that according to our findings, there is little sensational about these processes in wood-rotting fungi. They seem to follow the standard scheme, and show no feats which indicate a special adaptation to life in wood.

The slow gas exchange in wood cells will lead to an accumulation also of gaseous metabolic products, both from the living cells in standing trees, and from the mycelium. Of these, carbon dioxide is of course the most prominent. In conjunction with this, it is interesting to note the findings of Gundersen,[15] that CO_2 stimulated the growth of *Fomes annosus*. A growth stimulation by CO_2 is by no means unusual; it has been found in a number of bacteria, and from what we know about the role of CO_2 in metabolism, such as stimulation is also understandable. We lack, however, as we practically always do with these fungi, the experiments which would tell us which of the possible alternatives for the participation of carbon dioxide in the metabolism really operate in *Fomes annosus* and similar fungi. But what we may be permitted to do in this connection, is to point out that the growth stimulation by CO_2 may explain (at least partly) why *Fomes annosus* prefers living trees with high interior CO_2 content to felled timber, where the CO_2 has disappeared.

Inhibitory and stimulatory substances in wood

As already mentioned, wood has a very complex chemistry. This applies not only to the major constituents, but also to the number of organic compounds present in small amounts.

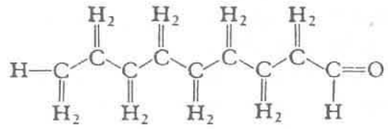

FIG. 10. The chemical structure of nonanal (pelargonic aldehyde).

In the heartwood of a number of tree species, one can find a whole lot of carbon compounds, mostly of aromatic character. Some of them are biologically inert as far as we know, but others are toxic to micro-organisms, and make up what often is called the heartwood toxins. Quite a number of such compounds are known, we represent them in Fig. 8 by a few of the better known, pinosylvin and its monomethyl ether from pine, and thujaplicin from *Thuja plicata*. These compounds act in an inhibitory way on wood-decomposing fungi, and may thus be regarded as a defence system. In fact, the heartwoods of some tree species are extremely resistent to decay. But as has been shown by Lyr,[16] at least some fungi can overcome this obstacle by detoxifying the heartwood toxins. It is thus not only the inherent toxicity of a given heartwood toxin that is of importance, but also the ease with which it is detoxified. The detoxification is not a specialized process. The fungus makes use of its oxidating enzymes, which have a low substrate specificity.

Lyr believes that among these peroxidase is of greatest importance, since it can make use of hydrogen peroxide which is formed by some of the respiratory enzymes, and the detoxification thus does not have to compete with the respiratory enzymes for the low supply of free oxygen present in the heartwood.

But this growth-inhibiting effect of the heartwood toxins, selective as it may be as to which fungi are able to grow in a certain species of wood, is only one side of the whole picture. The other side is represented by substances which promote growth. This is a very fascinating, but also quite elusive problem, which at present has only been clarified in small areas.

Cartwright and Findlay,[17] in their book *Decay of timber and its Prevention*, mention as an old observation that *Merulius*, when growing in a brick wall in which there is some constructional timber, may be seen to grow in the direction of the timber. This is a kind of growth response that one would call chemotaxis, and the rational explanation in this case would be that the fungus grows along the concentration gradient of a volatile compound emitted from the wood, which stimulates growth.

Suolahti[18] has studied this phenomenon in more detail, and has been able to show that at least in part, there is some real basis for this observation. He used the fungus *Stereum sanguinolentum*, and we see in Fig. 9 how it reacts on wood, even when there is no direct contact between the fungus and the wood. By comparative experiments with different chemical compounds, Suolahti reached the conclusion that it might be derivatives of fatty acids which were responsible for the effect. Later on, Fries[19] demonstrated that nonanal (pelargonic aldehyde) (Fig. 10), which can be formed by spontaneous decomposition of linoleic acid, gives the same effect. About the role of long-chain aldehydes in metabolism we do not know much. They act in the luciferin-luciferase system, but otherwise we have little information about their role.

Also in litter there are compounds that act on the growth of the Hymenomycetes. This has been shown in works from Melin's laboratory.[20] Recently, Lindeberg reported on growth-stimulating and growth-inhibiting compounds in freshly-fallen aspen leaves. The interesting feature in Lindeberg's findings is that the compound(s) (which are not yet identified) seem to act in a different manner on different fungi. The litter-inhabiting fungi on which they have been tried are stimulated, and the wood-decomposing fungi are inhibited.

To make the picture complete, we should also mention the compounds that are excreted from roots, and which seem to stimulate the mycorrhiza-forming Hymenomycetes to engage with the root cells.[21]

To sum up

Should we attempt to make a summing-up, and try to reach a conclusion on this topic, I would like to stress that there must of course be many quite different factors which are important to life of fungi in wood. The necessary enzyme equipment for digestion of wood must naturally be present. But it seems as if the reason why some Hymenomycetes prefer litter, while others enter the wood, perhaps for the greatest part is due to how they react on different chemical compounds that are found in small amounts in wood and in litter.

However, to clarify this is a tremendous task upon which work has barely started. First, these subtle compounds have to be isolated and identified, and then, their mode of action has to be clarified. In order to be able to do this, we need to know a whole lot more of Hymenomycete biochemistry and physiology than we do at present.

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